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CRITERIA FOR EDIBILITY OF OILS AND FATS

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CRITERIA FOR EDIBILITY OF OILS AND FATS

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Indian Standard

CRITERIA FOR EDIBILITY OF OILS AND FATS

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 15 September 1984, after the draft finalized by the Oils and Oilseeds Sectional Committee had been approved by the Chemical Division Council and the Agricultural and Food Products Division Council.

0.2 Most of the fats and oils in use today for edible purposes are traditional items that have become accepted for the purpose through centuries of usage. Sesame, rape, mustard and coconut oils are perhaps the oldest of these, followed by the Portugese introduction of the groundnut and its oil in the sixteenth and seventeenth centuries. Other local traditional edible oils are those of sunflower and niger, and in a smaller way of sal, *Dhupa*, *Kokum*, linseed, etc.

0.3 In recent times, new oils have been introduced whose edibilities were primarily established by usage elsewhere. Examples of such materials are refined cottonseed oil, sunflower seed oil, soybean oil, palm oil and tobaccoseed oil, which were therefore accepted without question in India as being edible.

0.4 Recently, however, new developments have occurred. Certain minor oils used in local diets have been commercialized, such as sal seed fat, mango kernel fat and *Kokum* butter. Other fats about which less is known are becoming available, such as the seed oils of the citrus family, water-melon, pompkin, tea, coffee and the like, whose edibility has not been traditionally established. Moreover, even fats that are known to have been consumed by human beings cannot, on that score, be automatically assumed to be beneficial to health on unrestricted consumption.

0.5 A certain protocol of testing is today in vogue to determine the edibility and toxicity or otherwise to human beings of any ingested product. Ideally this involves, stepwise examination of analytical characteristics, biological testing on rats, biological testing on two other animal species including a non-human primate, and final testing on human volunteers. Certain steps may be omitted, for example, if the fat is already in edible use, and rat tests are normal.

0.6 This standard outlines procedures for the stepwise examination of a fat or oil that is under consideration for edible use. The various steps and their significance is first outlined in general terms. This is followed by the broad experimental directions relating to each step.

0.7 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS : 2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

1. SCOPE

1.1 This standard outlines the stepwise criteria to be followed to determine the edibility or otherwise of an oil or fat.

2. STEPS INVOLVED

2.1 History of Use — Collect information from local practices or from historical records regarding usage of the oil or fat, either for internal consumption as a medicinal or edible material, for external application or for non-human usage. These data will offer some clues to the possible properties of the fat in its unrefined form. However, once the fat has been refined, these properties may not still apply. An inedible fat may become edible on refining.

2.2 Analytical Examination — Determine thoroughly the physical, chemical and spectral analytical characteristics of the fat, the nature of its component fatty acids and any unusual components of its unsaponifiable matter. Any unusual features will call for specific attention in animal testing.

2.3 Testing on Rats — The first biological testing is usually done on albino rats on diets containing 10 percent fats to determine chronic toxicity by way of fat digestibility, feed utilization, growth performance and indications of chronic toxicity. If the testing is continued for three generations, reproductive performance on the fat-containing diet can also be estimated. For acute toxicity, the fat as injected, and the organs examined histopathologically.

2.4 Testing on Other Animals — It is desirable to also test the fat for edibility on two other animal species, one of which is a primate that resembles man. The type of testing is similar to that in rats.

*Rules for rounding off numerical values (*revised*).

2.5 Human Testing — Fats found edible shall finally be tested by feeding to human volunteers, and the mass gain, fat digestibility and effect on serum lipids examined.

2.6 Determining Edibility — Putting together all the evidence from the experimental data, a statement should be possible regarding the edibility of the fat, and any restrictions indicated in respect of its unrestrained usage for human consumption.

3. ANALYTICAL EXAMINATION

3.1 Analytical Characteristics

3.1.1 Using standard method, determine the following usual analytical characteristics:

a) Melting point (if solid),

b) Refractive index,

c) Iodine value,

d) Saponification value,

e) Acid value, and

f) Content of unsaponifiable matter.

NOTE — Any oil or fat with a melting point of 45°C and above is not meant for direct edible consumption.

3.1.2 Examine the ultraviolet absorption spectrum (this will indicate any conjugated material) and the infrared absorption spectrum (for any unusual features).

3.1.3 Carry out the specific quantitative or qualitative tests for the presence of the following:

a) Hydroxy acid,

b) Epoxy acid,

c) Cyclopropene fatty acids, and

d) Any other toxic material, such as alkaloids, etc.

3.1.4 Determine the percentage of glycerol present.

3.2 Fatty Acid Composition — Examine the fatty acid components present in the form of their methyl esters, qualitatively by thin-layer

chromatography (TLC) and quantitatively by gas-liquid chromatography (GLC) or high pressure liquid chromatography. Where GLC is used, it is advisable to determine the composition both on short silicon columns and on long polyester columns. Where unusual fatty acids are present, the chromatographic methods should be suitably adopted. Separation of the total fat by silver ion-TLC against a known fat of similar unsaturation will also serve to indicate any unusual components.

3.3 Examination of Unsaponifiable Matter — Many classes of compounds like sterols, hydrocarbons, pigments, alcohols, alkaloids, etc, are present, each class being itself a complex mixture. Resolution by TLC of the unsaponifiable matter of the test fat, alongside that from a familiar material like groundnut oil, will point to any unusual constituents. Use of three solvent systems for separate resolutions will also be helpful. Such solvent systems using TLC on silica gel G are as follows:

a) Benzene alone,

b) Benzene : ethyl acetate (95 : 5), and

c) Chloroform : trichloroethylene (30 : 70).

3.4 Interpretation — The tests so far will show whether the fat is unlikely to be edible, for example, if the contents of hydroxy, epoxy or cyclopropene acids, or of unsaponifiable matter, are high, or whether further testing is justified. It will also indicate any special emphasis in the conduct of the animal work. Thus a saturated fat will call for specific attention in animal studies to digestibility, skin condition, tail necroses and the nature of serum and liver lipids. A fat containing cyclopropene fatty acids may be expected to lead to accumulation of stearic acid in adipose tissues. A high content of unsaponifiable matter, or unusual constituents, may call for special feeding studies with isolated unsaponifiable matter. Previous literature on the specific effects of certain constituents must be examined to further guide the course of animal studies.

4. TESTING ON RATS

4.1 Acute Toxicity — Typically, 6 male and 6 female rats are injected intraperitoneally with 0·5 ml of the test oil daily for 7 days, with groundnut oil as a control. All the animals are sacrificed and all the organs (liver, kidneys, testes and heart) are examined histopathologically for any abnormalities in comparison with the controls.

4.2 Long-Term Rat Studies

4.2.1 *Methodology* — As an example, 24 male and 24 female weanling rats are divided into two groups with equal numbers of either sex. Each

is fed for 22 weeks on a diet adequate in all respects containing 10 percent of the test oil in one group and 10 percent of groundnut oil in the other. The animals within groups are mated, and the feeding continued for three generations on the weaned animals.

4.2.2 *Growth Performance and Feed Efficiency Ratio* — For each generations, the gain in body mass for weeks is observed and the feed efficiency ratio is calculated separately for males and females.

4.2.3 *Fat Digestibility* — Faeces are carefully collected (adhering food must be removed) separately from each animal on say, the 10th day on the diet. The faeces are dried, ground and saponified with alcoholic potash, after which the soaps are acidified with dilute sulphuric acid, and the fatty matter isolated with light petroleum. A control group of rats on the same diets without added oil, but with the same mass of added sucrose (to make the diets isonitrogenous), serve as controls for fat excretion. The difference shows the extra fat excreted coming from the fat fed, from which the percentage digestibility is calculated for both the test oil and groundnut oil.

4.2.4 *Nature of Rat Lipids* — At the end of the first generation feeding, the skin condition is noted and the content of serum lipids and of liver lipids is measured. Additionally, if possible, the following determinations are also undertaken:

a) Serum and liver cholesterol and triglyceride levels;

b) Determination of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) spectra by serum ultracentrifugation, and of very low density lipoprotein (VLDL) by agarose gel electrophoresis;

c) Content of vitamin A in the liver; and

d) Fatty acid composition of serum lipids [with estimation of the ratio between 20 : 3 (n-9) and 20 : 4 (n-6) fatty acids], adipose tissue lipids and heart muscle lipids.

4.2.4.1 The animals fed groundnut oil serve as controls.

4.2.5 *Reproductive Performance* — Records are kept for the three generations of animals and the following are calculated:

a) The percentage of females who have conceived;

b) The mean number of days taken from IFM to delivery;

c) The average litter size and average birth mass; and

d) The average weanling mass and percent preweanling mortality.

4.2.5.1 The animals fed groudnut oil serve as controls.

4.2.6 *Chronic Toxicity* — Key organs of the body (liver, kidneys, testes and heart muscle) are examined histopathologically for any abnormalities in comparison with the groundnut oil fed animals.

5. TESTING ON OTHER ANIMALS

5.1 Choice of Animals — Complete toxicological evaluation calls for testing on rats and two other animal species, one of which is a non-human primate. Rabbits, hamsters, mice or dogs are possibilities for the first category and any species of monkeys would fall into the second.

5.2 Methodology for Other Animals— Principles are the same as for testing on rats, but shorter periods may be used. Thus for digestibility trials, 5 to 7 growing male animals may be kept for 7 days on the diet, and the faeces collected on the 8th day; or three groups of animals may be kept respectively on the test diet, a groundnut oil control diet, and a fat-free diet (with added sucrose) for say, 15 days and the faeces collected for the last 5 days; or the same group of animals may be kept in turn on the test oil diet, a fat-free diet, and a groundnut oil diet for say, a week each, and the faeces collected on the last day of the particular feeding. Acute toxicity trials may be carried out by fat injection, as described for rats.

5.3 Methodology for Monkeys — As an example, 2 groups of 8 monkeys (*Macacca radiata*), matched for body mass, are put on diets containing 18 percent protein and 10 percent of total fat (test oil or groundnut oil). After 2 months, the peripheral blood samples are drawn and analysed for lipid phosphorus, total cholesterol and triglycerides, and if possible for fatty acid composition. To determine any possible effect on lipid metabolism, the levels of enzymes such as glutamic oxalacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) may be determined. Also, after feeding for a period of 6 to 12 months, the animals can be sacrificed and key organs like the heart, liver, adrenals and sartorius muscles examined histopathologically after suitable staining.

6. TESTING ON MAN

6.1 Choice of Subjects — Volunteers, usually young men, must be found who agree in writing to participation in the feeding trials, which are limited to digestibility studies and examination of serum lipids for tryglycerides, cholesterol and total fatty acid composition. If possible various classes of plasma lipoproteins may also be determined.

6.2 Methodology — As many subjects are possible, say 6 to 12, are kept for a period of 7 days on a diet very low in fat, at the end of which the faeces are collected for a day and analysed for the mass of fatty matter (metabolic fat excreted). Next 10 percent of the test fat (ingested fat) is added to the diet and after another 5 days; the faecal fat excreted is again weighed. The percent digestibility is given by the following formula:

$$\text{Digestibility, percent by mass} = 100 \times \frac{\text{Fat ingested} - (\text{Fat excreted} - \text{Metabolic Fat Excreted})}{\text{Fat ingested}}$$

Blood samples at the end of the fat feeding period are drawn for analysis.

7. FINAL EVALUATION

7.1 Assembly of Data — The results of all the animal and human experiments, along with those of the controls employing groundnut oil, are compared, and a judicious conclusion regarding the edibility of the fat arrived at. This may be in terms of unrestricted human usage, usage with certain restrictions, or undesirability for use as an edible fat. Where the fats are used only occasionally, or in small amounts, the permissible criteria could also be more tolerant than for cooking fats used regularly and in volume. A further mitigating circumstances in India is that the invisible fat present in almost every dietary constituent, notably cereals and pulses, constitutes 10 to 20 grams every day, as is of a highly unsaturated nature with about 50 percent of linoleic acid.

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